An Evaluation of Methods for the Extraction of Dissolved Organic Compounds from Sea Water

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by

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ABSTRACT

Leonard, J.D., N.F. Crewe, and R. Pocklington. 1988. An evaluation of methods for the extraction of dissolved organic compounds from sea water. Can. Tech. Rep. Fish. Aquat. Sci. No. 1606: iv + 19p.

Although many extraction methods have been used for organic compounds in sea water, very few comparisons of these methods have been published. This document reports on an evaluation of three of the most common methods. Organic compounds were extracted using three different methods: adsorption on an XAD2 resin, direct solvent extraction in glass samplers and solvent extraction in a continuous flow extractor. The three methods each had positive and negative features. The XAD allowed for large volumes of water to be processed but appeared weak for simple aliphatic hydrocarbons. The continuous flow extractor was very efficient for nonpolar compounds but was unusable under moderate sea conditions. The glass bulbs were awkward to handle and limited samples to 12 litres but allowed for sampling at different depths and were suitable for many classes of compounds.

RESUME

Leonard, J.D., N.F. Crewe, and R. Pocklington. 1988. An evaluation of methods for the extraction of dissolved organic compounds from sea water. Can. Tech. Rept. Fish. Aquat. Sci. No. 1606: iv + 19p.

Quoique de nombreuses méthodes aient été utilisées pour l'extraction de composés organiques de l'eau salée, très peu de comparaisons de ces méthodes ont été publiées. La présente étude porte sur une évaluation de trois des méthodes les plus communes. On a effectué l'extraction de composés organiques selon trois méthodes: l'adsorption sur la résine de type XAD2, l'extraction directe du solvant dans des échantilloneurs de verre et l'extraction du solvant dans un extracteur à débit continu. Les trois méthodes ont montré des aspects négatifs et positifs. L'adsorption sur résine XAD2 a permis le traitement d'importants volumes d'eau mais montrait une certaine faiblesse dans le cas des hydrocarbures aliphatiques. L'extracteur à débit continu était très efficace pour l'extraction de composés non polaires mais était inutilisable en mer agitée. Les ampoules de verre étaient peu maniables et limitaient les échantillons à 12 litres; par contre, elles ont permis l'échantillonage à différentes profondeurs et étaient adéquates pour un grand nombre de classes de composés.

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INTRODUCTION

The analysis of trace organic constituents from the ocean requires highly specialized techniques. Because of the low levels of these compounds, large volumes of sea water must be processed in order to obtain sufficient material for even the most sensitive detection methods. In addition, any extraction method must operate with a high efficiency for the compounds of interest and must have a low risk of contamination by interfering compounds.

Several methods are possible for the initial step in the procedure - the water sampling. The water can be collected in sampling bottles or can be pumped on board the ship into collection devices. A third possibility is to use an in-situ system in which the sample is partially processed before return to the ship.

Once the water has been collected, several methods are available for extraction of the organic materials. One method which has been used extensively is sorption onto a solid matrix. Large volumes of sea water are passed through a solid absorbent which is later extracted with small volumes of solvent. Because the adsorbent is only in contact with the limited amount of water which is flowing through it at any time, large volumes of water can be extracted with a small amount of adsorbant. This adsorbant can then be extracted with a small volume of solvent.

Ratios of water extracted to solvent (and hence preconcentration of the extracted organic compounds) are quite commonly 500 and can be much higher. Limiting the volume of solvent also limits problems from solvent blanks.

One widely used solid adsorbent is Amberlite XAD-2 which is a macroreticular resin co-polymer of styrene and divinylbenzene that has been shown to combine high sorption capacity with excellent desorbability. It has been used effectively for the analysis of drinking water, waste waters (Junk 1974), sea water (Derenbach 1978), etc.

Another method which has been used for the extraction of organic compounds is solvent extraction. Although organic solvents of very high purity can be readily obtained, the main obstacle to be overcome is carrying out an effective extraction without using prohibitively large volumes of solvent. It is necessary to disperse the solvent in the water so that contact is made with the organic solutes and then to recover the largest possible proportion of the solvent.

One approach to solvent extraction is that of workers at the Deutsche Hydrographische Institut who used large (10-20 l) glass bulbs to collect seawater (Stadler 1977, Gaul 1983). The contents of these bulbs were extracted with small volumes of solvents. By using suitable attachments, the solvent was recovered even after it had been quite forcefully mixed into the seawater.

Another approach is to use a continuous flow liquid-liquid extraction system such as the Ahnoff-Josefsson extractor (Ahnoff 1974). This extractor enables seawater to be continuously extracted by a relatively small volume of solvent which is retained to the end of the extraction.

The aim of our study was to evaluate several of these methods for the collection and extraction of organic solutes and to assess some of the positive and negative attributes of each.

METHODS

The methods were evaluated by using them for the analysis of real samples. From a knowledge of what was present in the different extracts and, in some cases, what was present according to literature reports, it was possible to evaluate the different methods. Using this approach, it was known that the compounds were in the water in their natural manner rather than in a spiked situation where for nonpolar compounds one can never be certain that the spikes are well-distributed. By using natural conditions (rather than a laboratory simulation), it was also possible to observe how well these methods worked under various sea conditions.

The samples were collected from waters adjacent to our Institute (Bedford Basin) and on three different cruises: one on the Continental Shelf off Nova Scotia, one in the Baltic Sea (as

guests of Meereschimie, University of Kiel) and a third which included stations from the Continental Shelf off Nova Scotia across the Continental Slope to the Sargasso Sea . The most detailed evaluations were carried out on the first cruise where the XAD was compared directly to the Ahnoff-Josefsson extractor. The two later cruises involved the bulb sampler and its comparison to the XAD.

Several different methods were used for the collection of the samples. For most of the near surface (upper 3m) samples, water was pumped through FEP lines by an FMI pump with a teflon-ceramic piston chamber. Flow rates were in the range of 0.5-0.8 litres per minute. This flow was directed through precombusted glass fibre filters (Gelman AE, 1 um nominal pore size) to remove particulates and thence to the XAD-2 columns or an Ahnoff-Josefsson extractor. The DHZ bulb sampler was used at both 3m and at greater depths. To have a more direct comparison between the bulb sampling and the pumping, water was pumped into a bulb and this bulb was put through the normal bulb extraction procedure.

For the 3 m XAD samples, prefiltered water was pumped through the column at a flow rate of 400-700 ml per minute (5-7 bed volumes per minute) to a total of 50-150 litres. For the deeper samples, the seawater was collected in bulbs and pumped from them through the resin just as in the case of the shallow samples.

The XAD-2 was extracted for 12 hours in a Soxhlet apparatus with 80:20 acetone:water (Derenbach 1978). (Methanol was tried as a comparison to acetone but it was found that almost complete conversion of fatty acids to methyl esters occurred during the extraction of the resin). After concentration in a Kuderna-Danish apparatus which effectively removed the acetone, the water was extracted with residue-grade hexane, acidified and reextracted with hexane.

For the XAD-2 samples, one of the most important steps is the precleaning of the resin. It was exhaustively extracted (at least 24 hours) with an 80:20 acetone:water mixture until it produced a satisfactory blank and then stored in the same solvent mixture to prevent shrinkage of the beads or adsorption of contaminants. Even with this preparation it was found necessary to run blanks on the resin if it had been stored for any period of time, probably because of fracturing of the beads and bleeding from their interior. Fortunately, the material which bled was easily distinguished from the "real" signal.

Water for the Ahnoff-Josefsson extractor was collected in the same way as that for the XAD-2 column. Flow rate through this type of extractor was maintained at 35-40 ml/minute. 100 ml of hexane was used and recovery of the hexane at the end of the extraction was at least 95% by volume even when well over 100 litres of water had passed through the extractor.

Bulb samples were both collected and extracted in the bulbs.

Because of the high ratio of water to solvent using this method (10 1:100 ml) it is necessary that the solvent be extremely well dispersed in the water. At first the bulbs were shaken on an electric shaker but later it was found to be as effective to stir the mixture for ten minutes with a metal loop attached to a high-speed electric drill. In either case the hexane-water was allowed to separate and the hexane was removed by displacement with clean sea water. After removal of the first volume of hexane, the water was acidified to pH 1 and reextracted using the same procedure.

Total extracts after solvent concentration were divided and a fraction of each sample saponified using KOH/Methanol . Nonsaponifiables (sterols, alcohols, hydrocarbons, etc) and saponifiables (acids, etc) were collected separately by extraction with hexane before and after acidification. All hexane extracts were concentrated using a Kuderna-Danish apparatus and finally under nitrogen to volumes of less than one ml for GC and GCMS analysis.

Analyses were performed on a Finigan 4000 GCMS with an INCOS data system and on an HP 5730 GC with both FID and ECD detectors. Splitless injection onto 30m fused silica columns (OV-1) was used for all analyses. The ECD was used for only a limited number of the analyses.

RESULTS

1 Baltic Cruise (XAD-2 resin vs Ahnoff-Josefsson extractor)

Three stations were occupied and samples collected with XAD-2 and Ahnoff-Josefsson extractors. One of the A-J samples was lost, however, leaving a total of five samples.At each location, one stream of filtered water was split between the two extraction methods, and hence any differences in the organics obtained must be method dependant. The n-alkane distributions from the Baltic cruise are shown in Table 1. It is quite obvious that the continuous flow liquid-liquid extractor showed a much higher effectiveness for the extraction of aliphatic hydrocarbons. This is not unexpected as XAD-2 has been shown to have some weakness in the extraction of these compounds (Derenbach 1978).

In the A-J samples, the C13, C15 and C17 n-alkanes are predominant and the C15, C17 and C19 monoenes as well as the C21:6 isomers are observed at relatively high levels. All of these compounds are associated with naturally-occurring marine organic matter. C17 is predominant in many phytoplankton classes (eg Chlorophyceae, Rhodophyceae) as well as some benthic marine algae while C15 predominates in Phaeophyceae and marine bacteria (Saliot 1981).

Another group of compounds which are evident in the XAD extracts are the fatty acids. Although XAD-2 is reputed to be a relatively poor adsorber for fatty acids (Junk 1974), fatty acids, whether free initially or esterified in steryl and wax

esters and in phospholipids and triglycerides, constitute the largest signal in our analyses. Part of this signal may be due to very small particles which are able to pass the filter but which are trapped in the XAD-2 column.

Free fatty acids were identified in only one of the A-J (Baltic) samples and even then only at trace levels. This low level of free fatty acids may be due to the unfavourable partition coefficient between the hexane and the water. Shultz and Quinn (1972) report many of the fatty acids as salts at natural pH. It may also be tht the acids are able to travel through the Ahnoff- Josefsson on colloidal size particles.

A number of other classes of compounds appeared in either the A-J or the XAD-2. Most of these also appear to be from a natural marine source. These include a series of aldehydes (Table 3) in the A-J and a series of alcohols in the XAD-2 resin.

The alcohols are similar to the acids in that they are missed by the Ahnoff-Josefsson. Again, partition coefficients and adsorption on very fine particles are controlling factors. The aldehydes, being less polar, are more easily removed by the Ahnoff-Josefsson extractor. It is surprising that they were so poorly extracted by the XAD.

Methyl esters (Table 3) were observed, especially from the A-J samples. The most concentrated of these esters were the C12:0 and the C14:0 rather than the C16-C22 range with associated unsaturated compounds typical of natural marine matter. These

esters differ in source from the fatty acids. Again, being nonpolar, they are most efficiently extracted in the Ahnoff-Josefsson extractor.

In addition to the above classes of compounds, a number of substituted aromatic compounds were identified in the XAD-2 and to a lesser extent in the liquid-liquid extraction. The most widely distributed of these compounds are listed in Table 2. XAD-2 is reported to have its highest efficiency for aromatics, and especially for condensed ring aromatic compounds; however, the differences reported here were more probably related to the volumes of water which were processed. Because the XAD-2 could handle approximately 10 times the flow of the liquid-liquid extractor, it is reasonable that the signal would rise above background more often with the resin. The traces of nonyl phenols in the A-J samples, for example, might have been missed entirely had those compounds not been present in larger quantities in the XAD.

The nonyl phenols observed were identified from their mass spectra and the pattern of their distribution as compared to that shown by Giger et al (1981). No evidence was seen of the nonyl phenol ethoxylates reported by Giger but we have no information on the extractibility of these compounds. The nonyl phenols observed are almost certainly anthropogenic in nature, but since they are used in a number of industrial formulations their exact source is unknown.

When the Baltic samples were analyzed with an ECD, both alpha and beta hexachlorocyclohexane were identified. The concentration of these compounds (alpha=0.5-2.6ng/l, gamma=0.8-4.3) was of the same order as that found by Brugman (1982) in the same area. No other chlorinated organics were detected although a trace of hexachlorobenzene was observed in the filters which were used in line with the extractors.

Thus, for these samples, it was observed that both the XAD-2 and the liquid-liquid extractor were effective in extracting organic compounds. The XAD-2 had the advantage that within a given period of time a much larger (X10) sample of the water could be processed. This meant that for the compounds which were present at extreme trace levels the possibility of observing a signal above background was much higher. The XAD-2 appeared to be relatively weak in adsorption power for aliphatic compounds as exemplified by the n-alkanes and the aliphatic aldehydes.

The Ahnoff-Josefsson extractor had its greatest advantage with nonpolar solutes; it was poorest for highly polar compounds. Because less water is processed with this extractor, it is obvious that this extractor is most effective for compounds at higher concentration or those for which a highly sensitive detector (such as ECD) can be used.

2) Scotian Shelf Bedford Basin (Bulb sampling)

With 12 l bulb samples from stations along the Scotian Shelf,

the major classes of compounds observed were the typical naturally-occurring marine lipids. The fatty acids corresponded very well with those reported for plankton and algae of sea water. The alkanes again showed a natural marine source with pristane being the largest individual alkane.

Because the bulbs collect the particulate phase as well as the dissolved, there is no possible distinction between particulate and dissolved organics. The compounds extracted appear to relate very closely to the organisms living in the water and to detrital matter from these organisms. Because of the limit in sample size (approximately 12 l), compounds present at extremely low levels are probably not detectable with the bulbs. Even with the 25 l bulbs which have been used in some studies not enough water may be collected to analyze compounds which are at extremely low concentrations. Lower levels can be measured, of course, for those compounds which use the most sensitive detectors such as the ECD.

3) Shelf/Sargasso Sea Cruise (AJ vs XAD vs bulbs)

These samples were taken on a cruise which was investigating the organic matter changes as one passes from the Continental Shelf to the Continental Slope to the Sargasso Sea.

The only major difficulty which occurred with any of the methods was with the Ahnoff-Josefsson extractor. Because the ship motion was quite strong, the vortex produced by the stirring

bar was often disturbed and, on occasions, was lost completely and had to be restarted. This was a considerable nuisance since it required that the extractor be watched continuously.

With the XAD and bulb sampling the signal variation from station to station and from depth to depth could be attributed to variations in phytoplankton productivity and in activity of zooplankton. Because the bulb sampling uses the total water sample while the XAD-2 works on a prefiltered sample the levels were, of course, different in the two methods.

Qualitatively, there was a good agreement between the bulb samples and the resin samples. Both of these types of samples agreed very closely in fatty acid distribution to each other and to the filters. The bulb and the filter are both trapping particulates but the XAD is only trapping material which has passed through the filter. The distinction between dissolved and particulate is not a clear one, however, and the XAD may be trapping very small particles which have passed the filters. These may be debris from the larger particles. Much of the material in all of the samples is dead material as shown by the complete lack of polyunsaturated fatty acids characteristic of fresh marine organisms.

As in the Baltic, the XAD appeared to be weaker towards alkanes than the bulb extraction, even when the levels in the filter were considered. The results were not as clear, however, because of lower levels overall.

One other observation from this cruise pointed out the care which must be maintained at all times. For several samples, water was pumped using the BIO insitu pump and unfortunately this water was found to be highly contaminated with phthalates, almost certainly originating from the plastic tubing used on the pump in this system.

CONCLUSIONS

Each of the methods of this study was used in several very different situations. They all were effective in extracting organic solutes but each has its own advantages and disadvantages.

The glass bulb sampler is an excellent system for the recovery of a wide range of compounds. The bulbs can be awkward to handle and do limit sample size, unless multiple samples are taken. They can be deployed at depths to several hundred metres to allow for samples at depth. Once recovered, they do yield high recoveries for a wide range of compounds. They also give the possibility of a second extraction after acidification. No distinction is made between dissolved and particulate.

XAD-2 allows a very large sample to be processed and hence it is possible to look at compounds at much lower concentration levels. The water must be pumped Most of the problems with

XAD-2 arise from the initial cleaning of the resin and the maintaining of this cleanliness

The A-J liquid-liquid extractor is very effective for the extraction of lipophilic compounds. However, because the flow is limited to 2-3 l/hour, long sampling times are required if one is interested in compounds at trace levels. The A-J is also suspect under conditions of high swell where its vortex is lost easily. For this reason it seems more suitable for longterm pumping on a more stable platform or in its submersible variation.

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Table 1 Comparison of XAD-2 and Ahnoff-Joseffsson extractor for n-alkanes in Baltic samples.

CARBON NUMBER	Stn1 XAD ng/l	Stn1 A-J ng/l	Stn2 XAD ng/l	Stn3 XAD ng/l	Stn3 A-J ng/l
11	--	26.7	--	--	6.8
12	--	20.4	--	--	6.9
13	--	38.5	--	--	12.3
14	--	***	--	--	***
15	.23	190.9	--	--	24.8
16	.42	12.3	.05	--	5.8
17	.83	34.6	.05	.05	12.4
18	.55	6.3	.08	--	6.0
19	.32	5.3	.05	.11	4.6
20	.28	3.1	.03	.03	3.3
21	.34	5.3	.07	.20	5.9
22	.49	4.8	.27	.19	6.2
23	.49	5.6	.16	.16	7.0
24	.51	3.4	.22	.17	6.0
25	.63	4.1	.22	.18	6.1
26	.54	3.1	.44	.25	13.1
27	.48	2.5	.49	.40	13.0
28	.21		.44	.25	6.2
29	.17		.24	.24	7.7
30			.11		2.0
TOTAL	6.5	366.9	2.9	2.2	156.1

(-- below quantitation limit; *** unquantifiable because of coeluting peak)

Table 2 Nonyl phenols and other aromatics in Baltic samples.

	01-XAD	01-AJ	02-XAD	03-XAD	03-AJ
Nonyl Phenols					
1			.03		
2	.015		.06	.021	.124
3	.012	tr	.056	.007	.093
4	.007	tr	.131	.007	.124
5			.024		
6	.004	tr	.098		.139
7			.066	.011	.015
8			.080		
9	.004	tr	.158	.014	.046
10			.012		
11			.024		
TOTAL	.04		.74	.06	.54
Poyaromatics					
Metnylquinoline			pres	pres	
Dimethylquinolines			4 pres	1 pres	
1H-Indole	pres			pres	pres
Naphthalene	pres	pres		pres	pres
Dimethylnapth.	2 pres	2 pres		2 pres	2 pres
Trimethylnaphth.	pres			2 pres	pres
Fluoranthene			pres	pres	pres
9,10-phenanthrene-dione	pres		pres	pres	pres

pres-compound observed but not quantified
tr-only at trace levels

Table 3 Hexachlorcyclohexane isomers, methyl esters and aldehydes in Baltic samples

	Stn1 XAD ng/l	Stn1 A-J ng/l	Stn2 XAD ng/l	Stn3 XAD ng/l	Stn3 A-J ng/l
HCH isomers					
alpha	0.7	0.5	2.6	0.9	1.2
gamma	1.8	0.8	4.3	1.2	2.4
Methyl Esters					
C12:0	-	24.8	-	-	8.7
C14:0	-	7.4	-	-	2.9
C16:2+3	-	0.3	-	-	0.7
C16:1	-	-	-	-	2.9
C18:2+3	-	-	-	-	0.5
C18:1	-	-	-	-	0.5
C18:0	-	0.2	tr	tr	0.6
Aldehydes					
C13	-	3.5	-	-	2.2
C15	-	2.0	-	-	4.9
C16	-	1.6	-	-	2.3
C17	-	4.5	-	-	5.4